

The University of Chicago

HISTAMINE AS A CONSTITUENT OF SECRETIN PREPARATIONS

A DISSERTATION
SUBMITTED TO THE FACULTY
OF THE OGDEN GRADUATE SCHOOL OF SCIENCE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY

BY
ELOISE PARSONS

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From the Hull Laboratories of Physiological Chemistry and Pharmacology

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Secretin action is found in a great many extracts and tissues. Acid extracts of duodenum, jejunum and part of the ileum, according to Bayliss and Starling (1), and confirmed by many others, have a very great secretagogue action on the pancreas, when injected into an animal. Acid maceration of mesenteric ganglion of the rat causes secretion from the pancreas, according to Camus (2). The presence of secretin in aqueous extracts of liver, thyroid and thymus, as reported by Rodgers, Rahe, Fawcett and Hackett (3), is open to criticism in that the effect produced may have been secondary to the action of a primary gastric stimulation. Luckhardt, Henn and Palmer (4) show that secretin prepared from the gastric mucosa is as effective as secretin from the duodenal mucosa in producing a secretion from the pancreas. Witte's peptone in some cases is reported to cause a secretion of pancreatic juice. Van Eweyk and Tannenbaum (5) report that casein or egg white digests have no effect until they are heated to 280°C., when they show good secretagogue action. Amino acids, according to Sweitzer (6), have no secretagogue action, but van Eweyk and Tannenbaum find that of all the amino acids, histidine alone when heated to 280°C. gives a substance which, when injected, causes the pancreas to secrete. Secretin preparations, however, heated to 280°C. lose their activity. Van Eweyk and Tannenbaum, also, confirm the finding of Bickel (7) that there is a substance in spinach which has a marked action on the flow of pancreatic juice. This spinach secretin they claim to be free from depressor action.

Voegtlin and Meyers (9) consider the antineuritic vitamine to have secretagogue action, but Anrep and Drummond (10) show that it is not identical with secretin. Uhlmann (11) found that the commercial vitamine "orypan" stimulated pancreatic secretions when injected intra-

venously, subcutaneously or by oral administration into a rabbit. He also finds the activity distributed in rice polishings, spinach, nettles, meat, oats, yeast, clover and cabbage. Popielski (12) considers that all these substances have in common "vasodilatin" which acts to cause a lowering of the blood pressure, a decrease in the coagulability of the blood, a flow of gastric and pancreatic juices, and of saliva, the general similarity of the action being due to the probable splitting of the substance with the liberation and the subsequent action of vasodilatin, which, he suggests, is in all probability due to an imidazol derivative, possibly histamine or a "histamine-like" substance.

The fact that secretin activity is found in so many different tissues suggests that the action is due either to a variety of substances which are rather widely distributed, or if it is due to a single substance, this must be a constituent of a great variety of materials.

Abel and Kubota (13) were of the opinion that histamine is a rather widely distributed substance, everywhere the same, which may have its origin in various tissues, and which makes its appearance wherever protoplasm is killed. In other words, it arises wherever a true protein is even partially disrupted by enzymes, acids or other hydrolytic agents. They obtained histamine from hypophysis, Witte's peptone, ereptone, casein (Kahlbaum) and edestin by acid hydrolysis, as well as in an acid extract of gastric mucosa and duodenal mucosa. The substance which they isolated in each case formed a picrate which melted at 236 to 237°C., gave a strong Pauly reaction, was precipitated by phosphotungstic acid and by mercuric chloride. It yielded a crystalline gold chloride and platinic chloride. As a free base, it is soluble in water, alcohol and hot chloroform, but insoluble in dry ether. These are the properties of histamine; hence their substance was identical with histamine in crystalline form, melting point and solubility. Moreover, the physiological behavior of the two showed their substance to be identical with histamine in the lowering of the blood pressure in a cat, and the contraction of the virgin guinea pig uterus.

Much of the work of Abel and Kubota has been disproved by the determination of histamine by the more accurate method devised by Koessler and Hanke (15). These workers show that hypophysis which has not been acted upon by bacteria contains no histamine. Peptone prepared under sterile conditions contains no histamine, but is capable of producing a typical peptone shock. An analysis of pure casein hydrolyzed by acids, shows no histamine, but the pharmacological findings verify those of Abel and Kubota, in that there is a fall in the blood pressure when it is injected intravenously into an animal. The conclusion is, that there is a substance present in the peptone and hydrolyzed casein, which behaves similarly to histamine, but which is not identical with histamine.

Barger and Dale (14) obtained from the mucosa of the small intestines of an ox, a picrate quite similar to histamine picrate, the melting point of which was 232°C . It gave the Pauly reaction for imidazol and physiologically caused a fall in blood pressure and a flow of pancreatic juice. They state, however, that the substance has less power to produce pancreatic juice than a dose of secretin which depresses to the same amount.

The development of the accurate chemical method of Koessler and Hanke (15) for the determination of histamine in protein and protein containing matter, makes possible the accurate determination of histamine in secretin solutions. The method is specific for histamine; other substances which are similar are eliminated in the process of the analysis. When a protein containing extract is analyzed by this method and found to contain histamine, then, if the activity is similar qualitatively and quantitatively to histamine, that activity may be said to be due to histamine, provided the quantity present is comparable with an amount of pure histamine which produces the same effects.

Secretin, according to one prevailing opinion, owes its activity to histamine. It was thought that by determining the histamine present in a secretin preparation, and by comparing these results with the determinations on secretin preparations to which known but reasonable amounts of histamine had been added, one might throw some light on the claimed identity of histamine and secretin.

EXPERIMENTAL: Preparation of secretin. The duodenum and part of the jejunum were taken from dogs, killed just previously by bleeding. This material was washed thoroughly in cold tap water, dried between towels, the mucosa scraped off, ground and weighed. It was then dropped into hot 0.4 per cent hydrochloric acid solution, using 4 cc. for every gram of scrapings. The mixture was stirred while being brought to a temperature of 90 to 95°C . and allowed to stand over night. The next morning it was again brought to 95°C . and filtered. The filtrate was concentrated under diminished pressure, until 1 cc. was equivalent to 4 grams of the original scrapings. The concentrated secretin was neutralized with Na_2CO_3 and filtered, the neutral filtrate being used for the experiments. In the preparation, it was less than 30 minutes from the death of the animal till the material was in the hot acid.

Histamine. Purified histamine dihydrochloride was used. This was a synthetic product, which contained 94.8 per cent histamine $(\text{HCl})_2$ and 5.2 per cent of NaCl and H_2O together. A stock solution was made by dissolving 2.1097 grams in water, diluting to exactly 200 cc. From this a solution was made by diluting 1 cc. of the stock solution to 100 cc. Each cubic centimeter contained 0.0001 gram histamine dichloride.

Estimation of histamine. The method of Koessler and Hanke (15b) was followed exactly. The material was in the liquid form, so that the

excessive humus formation noted by Gerard (20) in the acid hydrolysis was minimized.

Briefly, the method consists of 1, hydrolysis of the material in 20 per cent HCl for 30 hours; 2, removal of the hydrochloric acid by distillation in vacuo; 3, removal of the ammonia from alcoholic solution in which there is an excess of lime, by distillation in vacuo; 4, removal of the humin by filtration; 5, precipitation of the hexone bases from an acid solution by phosphotungstic acid; 6, decomposition of the precipitate by saturated barium hydroxide; 7, extraction of the histamine with amyl alcohol from a strongly alkaline solution; 8, recovery from the amyl alcohol by an acid solution and neutralization of the acid with barium hydroxide; 9, precipitation of the histamine by silver nitrate and barium hydroxide; 10, final purification of the histamine by means of chloroform.

The histamine fraction thus obtained is estimated colorimetrically by a modification of the Pauly reaction in which the amounts of reagents, sodium nitrite and sulphanilic acid are accurately measured, as well as the time during which they react. The alkalinity is carefully controlled also. The color is compared with a standard which is a mixture of congo red and methyl orange and which matches the color formed in the reaction of histamine perfectly.

In the preliminary work with this method, it was found that the decomposition of the phosphotungstate formed by the barium hydroxide required considerable attention. Unless the precipitate was triturated with $\text{Ba}(\text{OH})_2$ quite thoroughly in a mortar and unless the digestion was over a long period of time, some of the undecomposed precipitate remained. Since this is the first step in the analysis in which the secretagogue activity disappears, it was thought that the Van Slyke acid decomposition method might eliminate this loss of secretagogue reaction in secretin preparations. The analysis for histamine using both methods shows that the acid decomposition of the precipitate is as efficient as the alkaline for histamine, but the physiological tests for secretagogue action of secretin were quite negative by either method.

The report of a trace of color means that there was a color formed, but it was impossible to estimate the amount. The samples which were concentrated to 1 cc. gave a color reading. The color which appeared, but which was not on the scale of the colorimeter appeared at the same time after the addition of the reagents as the color for histamine and was the same shade as colors formed by histamine. The color readings are corrected for the colorimeter used and also by the factor for the reagents given by Koessler and Hanke. The equivalent amounts of histamine are taken from the tables prepared by the same authors.

Analysis. Five different secretin preparations were analyzed for histamine.

Experiment 1. Secretin "6" was a very active preparation, 1 cc. injected intravenously caused a fall in blood pressure of 60 mm. Hg and 36 drops of pancreatic juice in 10 minutes.

In the preparation of secretin, a gummy residue formed when the material was concentrated. This was usually filtered off and disregarded, but with this particular preparation it was saved and the histamine content determined. When the preparation was neutralized there always formed a protein precipitate which was filtered off. This was also saved and analyzed.

Two 100 cc. portions of the concentrated secretin were analyzed for histamine. Two other 100 cc. portions were analyzed, to each of which 1 mgm. of pure histamine dihydrochloride had been added.

Quantitative amount of histamine in secretin "6". One hundred cubic centimeters secretin during the analysis process was concentrated to 5 cc. One cubic centimeter of this gave a corrected reading of 35.4 on the colorimeter, which is calculated from tables to contain 0.0000474 gram of histamine. This makes 0.0002379 gram in the total 100 cc. analyzed. Duplicate analysis confirmed the result.

One hundred cubic centimeters of the same secretin preparation to which 0.001 gram pure histamine was added, was concentrated in the process of analysis to 25 cc., 0.5 cc. of which gave a colorimetric reading of 20.1, equivalent to 0.000025 gram histamine or 0.00125 in the total sample.

There was merely a trace of color from the gummy residue formed in the process of concentration.

There was only a trace of color from the precipitate formed when the secretin was neutralized.

The mean from the analysis above is:

0.00125 gram	histamine from 100 cc. secretin plus 0.001 gram pure histamine (HCL) ₂
0.000234 gram	histamine from 100 cc. secretin above
0.001016	= histamine recovered = 101.6 per cent

The amount of histamine present in 100 cc. secretin was 0.23 mgm.; the amount in 1 cc. which had strong physiological action when injected intravenously, was 0.0023 mgm.

The recovery of histamine shows that the technique and the method are satisfactory. Negative findings on the material which is being disregarded shows that no histamine is being discarded when the secretin is being prepared from the intestinal mucosa.

Experiment 2. Secretin "7" was a very active preparation which was obtained from the Department of Physiology. One cubic centimeter when injected intravenously into a dog caused a fall in blood pressure of 55 mm. of mercury and the flow of 16 drops of pancreatic juice in 10 minutes.

A 10 cc. portion was analyzed and the final solution concentrated to 10 cc. One cubic centimeter of this gave a color which appeared at its maximum intensity 4 to 5 minutes after the addition of the reagents, and which was the same shade of color as obtained when histamine was present, but the color was not intense enough to be on the scale of the colorimeter used.

The conclusion is that there was a *very* small amount of histamine present in 10 cc.

Experiment 3. Secretin "8" was a preparation from the Department of Physiology which had active secretagogue action as compared with a small depressor action. There was a fall in blood pressure of 30 mm. Hg when 4 cc. were injected intravenously, but a flow of 24 drops of pancreatic juice, in 10 minutes.

A 10 cc. portion of this was analyzed and the final solution concentrated to 10 cc. One cubic centimeter of this failed to give a color intense enough to be read on the colorimeter.

Experiment 4. In the preparation of secretin "9", the intestinal mucosa was digested in hot alcohol which contained 0.4 per cent HCl, the alcoholic extract being evaporated and the further preparation being as usual. In this preparation, the mucosa was in the hot alcohol about 15 minutes after the death of the animal.

Duplicate analyses were made on the secretin, using a 25 cc. sample in each case, and duplicate analyses were made on 25 cc. samples to each of which 0.001 gram histamine (HCl)₂ had been added.

The 25 cc. portion analyzed concentrated to 5 cc. gave just a trace of color formation. Concentration to 1 cc. or 0.5 cc. would have given a color which would have been on the scale of the colorimeter.

Another 25 cc. portion to which 0.001 gram histamine had been added was carried through similar analysis. Five-tenths cubic centimeter gave a corrected color reading of 15.8 which is equivalent to 0.000021 gram of histamine or 0.00105 in the total 25 cc. sample of secretin. Duplicate analysis gave 0.00110 gram histamine in total sample. A mean of the two is 0.001075 gram histamine.

A check analysis was made on 0.001 gram pure histamine in 10 cc. water. Five-tenths cubic centimeter of this gave a color reading of 38.0, equivalent to 0.00005 gram of histamine or 0.001 gram in the 10 cc. sample.

If the recovery of histamine is taken as 100 per cent as found, then the amount of histamine in 25 cc. of the secretin sample, according to 3, 4 and 5, is 0.000075 gram or 0.30 mgm. in 100 cc. of preparation "9". This is nearly of the same order as found in no. 6 which was 0.234 mgm. in 100 cc.

Experiment 5. Preparation 10 was made according to the usual method.

Only 10 cc. samples were used for the analyses, and only 0.1 mgm. histamine added for the control experiments.

In this analysis the phosphotungstate precipitate was decomposed by the Van Slyke acid method in one sample and by the usual alkaline method in the other. The results show that histamine could be recovered equally well by either method.

Ten cubic centimeters secretin were analyzed by the same method which has been used in all the samples analyzed, in which the phosphotungstate precipitate is decomposed by alkali. Concentrated to 1 cc., 0.5 of which gave a corrected colorimetric reading of 6.7, which is equivalent to 0.000009 gram of histamine or 0.000018 gram in the total sample.

Ten cubic centimeters of the same secretin preparation, in which the phosphotungstate precipitate was digested by acid according to the method of Van Slyke gave a value of 0.000016 gram histamine (HCl_2) in the total sample.

Ten cubic centimeters of the same secretin preparation to which 0.0001 gram of pure histamine had been added were analyzed, using alkaline digestion of the phosphotungstate precipitate. Five-tenths cubic centimeter gave a colorimetric reading of 4.5, equivalent to 0.000006 gram histamine or 0.00012 gram in the total sample.

A duplicate of the sample analyzed above was carried through the process with acid digestion of the phosphotungstate precipitate. Five-tenths cubic centimeter gave a corrected colorimetric reading of 4.3, equivalent to 0.0000055 gram histamine, or 0.00012 in the total sample.

One-hundredth gram histamine analyzed with the usual alkaline digestion of the phosphotungstate precipitate gave a recovery of 0.0096 gram of histamine.

One-hundredth gram histamine analyzed using the acid digestion of phosphotungstate precipitate, showed a recovery of 0.0092 gram histamine.

In this sample of secretin.

$$\begin{array}{ll} 0.00012 \text{ gram} & \text{histamine} = \text{amount in 10 cc. secretin plus 0.0001 gram pure} \\ & \text{histamine} \\ \hline 0.000017 \text{ gram} & \text{histamine} = \text{amount in 10 cc. secretin above} \\ 0.000103 = 103 \text{ per cent} & = \text{histamine recovered} \end{array}$$

The presence of 0.000017 gram in 10 cc. is equivalent to 0.00017 in 100 cc. which compares favorably with the 0.00023 gram found in secretin preparation "6", and the calculated amount, 0.0002 in secretin preparation "9".

The two methods of decomposition of the phosphotungstate precipitate were used in order to see if the loss of the secretagogue action of secretin could be prevented by the acid treatment. In each case all the depressor substance was precipitated by the phosphotungstic acid, and was re-

covered equally well by acid and by alkaline decomposition. The secretory activity could not be detected in the precipitate, nor did it remain in the filtrate. Comparable evaporations were made in every case so that the amount injected was comparable with the original secretin.

SUMMARY OF RESULTS. There is a very small amount of histamine present in secretin preparations.

That this amount of histamine is not enough to be of physiological importance is shown in the experiments which follow.

TABLE 1

Summary of quantitative estimation of histamine in secretin preparation

PREPARATION NUMBER	HISTAMINE FOUND	HISTAMINE IN 1 CC.
	<i>gram</i>	<i>gram</i>
6	0.000234 (in 100 cc.)	0.0000023
7	Trace (in 10 cc.)	
8	Trace (in 10 cc.)	
9	0.000075 (in 25 cc.)	0.000003
10	0.000017 (in 10 cc.)	0.0000017

PHYSIOLOGICAL ACTIVITY. In the same preparations which were analyzed chemically for the presence of histamine, the physiological action was followed. The action of the original preparation when injected into animals was compared with the actions of the different fractions obtained in the process of analysis, and finally with the final solution upon which the colorimetric estimation of histamine was made.

Preparation of animals for physiological experiments. Healthy dogs were used. An hour before the experiment the animal was given 0.25 gram barbital per kilo body weight. This was suspended in 50 cc. of water and put into solution with as little Na_2CO_3 as necessary. It was administered to the dog by stomach tube. The dog goes to sleep, and in most instances is so analgesic at the end of 30 to 45 minutes, that the operative procedure can be done without further anesthesia. In some dogs it is necessary to administer a small amount of ether at the time of the incision, but no further administration is necessary.

It has been found that under this anesthesia, the blood pressure maintains a high level, the respiration is slow and regular, and the secretory functions respond normally. This eliminates the variations in the blood pressure which are constantly present under ether anesthesia and the inhibition of gastric and pancreatic secretion so often observed under ether.

The animal was prepared by ligature about the pylorus, and a cannula in the pancreatic duct. Tracings were made registering the respiration,

blood pressure from the carotid artery, and the number of drops of pancreatic juice. Injections were made into the saphenous vein.

Table 2 shows the results obtained from secretin preparation "6".

Discussion. The results show that secretin "6" is a very active preparation, causing a fall in blood pressure of 68 mm. Hg and 36 drops of pancreatic juice in 17 minutes. After this has been digested with 20 per cent HCl for 30 hours, the fall in blood pressure is only 46 mm. Hg and there are only 3 drops of pancreatic juice in 10 minutes. When the material is treated with lime in an alcoholic solution to remove ammonia, there is no further loss in depressor or secretagogue action.

TABLE 2

Physiological activity of fractions obtained in analysis of secretin no. "6" for histamine

INJECTIONS	BLOOD PRESSURE					PANCREATIC SECRETION		
	Normal	Lowest reached	Fall	Time for return		Latent period	Drops juice	Time
				min- utes	seconds			
1 cc. concentrated secretin "6".....	116	48	68	17	2	93	36	17
1 cc. after acid hydrolysis for 30 hours.....	110	64	46	2	15	50	3	10
1 cc. after removal of am- monia.....	90	42	48	12		50	3	10
1 cc. decomposed P. T. A. precipitate.....	100	50	50	2				
1 cc. residue from above.....	108	No action						
1 cc. after extraction with amyl alcohol.....	96	50	46	2	15			
1 cc. residue.....	100	No action						
1 cc. after Ag precipitation final solution.....	106	78	28	0	27		Histamine con- tent 0.0023mgm. per cubic centi- meter	

After precipitation with phosphotungstic acid and decomposition of the precipitate, there remains a depressor action of the same order, but there is no secretagogue effect. Acid decomposition produces the same results, as stated before. In the solution from which the hexone bases were precipitated, there was neither depressor action nor secretagogue action. Somehow, in the treatment, the secretagogue material was destroyed or lost. Amyl alcohol removes the depressor substance from an alkaline solution, but in this process the secretagogue action is also lost. The final solution has a depressor effect on the blood pressure causing it to fall about one-half as much as the original secretin, but there is no secretagogue action remaining.

The results in table 3 are to be compared with those obtained from a secretin preparation to which a known amount of histamine was added. The fractions are similar to those in table 2.

The results above are to be compared with those obtained with histamine alone, when it is carried through the same process for quantitative analysis.

Injection of 0.0001 gram of histamine caused a fall in blood pressure from 104 mm. Hg to 40, a drop of 64 mm. Hg which did not return for 5 minutes 15 seconds. After a latent period of 2 minutes 34 seconds there were 6 drops of pancreatic juice which flowed in a period of 10 minutes.

After the removal of the ammonia, which occurs in the process of analysis, the histamine solution was injected and the effects noted. The blood pressure fell from 102 to 34 mm. Hg and returned to normal after a

TABLE 3
Physiological activity of secretin fractions to which histamine has been added

INJECTION	BLOOD PRESSURE					PANCREATIC JUICE		
	Normal	Lowest reached	Great-est fall	Time for return		Latent period	Drops juice	Time
				min-utes	seconds			
1 cc. secretin with 0.1 mgm. histamine.....	106	32	72	13	15	45	43	10
1 cc. after removal of ammonia.....	104	34	70	7	30	45	8	10
1 cc. after amyl alcohol treatment.....	106	40	60	5		92	5	10
1 cc. after Ag precipitation....	100	38	62	2	30	90	4	10

period of 6 minutes. There was a flow of 6 drops of pancreatic juice after a latent period of 2 minutes 30 seconds.

When the histamine-containing material was injected after precipitation with phosphotungstic acid, the blood pressure fell from 108 mm. Hg to 48, a fall of 60 mm., which returned in 3 minutes 50 seconds. There were seven drops of pancreatic juice which flowed for a period of ten minutes following a latent period of 2 minutes 40 seconds.

The treatment with amyl alcohol had no deleterious effect on the physiological action. After treatment with amyl alcohol the histamine-containing solution injected caused a fall in blood pressure from 106 to 44 mm. Hg, a lowering of 62 mm. which returned to normal after 4 minutes 30 seconds. There were 5 drops of pancreatic juice which flowed in ten minutes following a latent period of 2 minutes 35 seconds.

The final precipitation with silver left a solution which caused a fall

in blood pressure from 100 to 44 mm. Hg. This drop of 56 mm. was recovered from in one minute 10 seconds. The injection also caused a flow of 5 drops of pancreatic juice after a latent period of 2 minutes 40 seconds.

Discussion. Histamine causes a flow in pancreatic juice, but only about one-sixth as much as a secretin preparation which depresses to the same amount. The secretagogue action of histamine is as strong in the final solution as in the original, a flow of 6 drops being observed in the original, as compared with 5 drops in the final solution, in the same period of time. In the secretin solution to which histamine had been added (table 3) the result of the injection of the two together seems to be the additive effect of their individual actions. After the precipitation of the hexone bases by phosphotungstic acid, and the treatment of the alkaline solution with amyl alcohol, the effect due to the secretin disappears, and the results are similar to those obtained with histamine alone.

Similar results were obtained in the other preparations of histamine analyzed.

SUMMARY OF RESULTS. 1. Secretin loses its ability to cause the pancreas to secrete during the process by which it is analyzed for histamine.

2. Histamine, which has a lower, but nevertheless decided secretagogue action, loses none of it by the same process.

3. Secretin, to which histamine has been added, when thus treated, loses the activity due to the secretin, but retains the activity due to the histamine.

4. The depressor action is lessened after the analysis in the case of secretin, but is still present. It is but slightly lessened in histamine.

INSTABILITY OF SECRETIN. It is not surprising that the results of the vigorous chemical treatment involved in the analysis for histamine leave a material which shows no secretagogue activity, in view of the instability which many secretin preparations exhibit. Some preparations of secretin in acid or neutral solution retain their activity for months, others lose theirs in a few days, even though they are kept under the same conditions, apparently.

The results (table 4) are taken as being illustrative of the instability. A sample of each preparation of secretin was kept in a sealed tube which had been sterilized by bringing it to a boil on three successive days. The stock solution was covered with a layer of toluol and kept in the ice box.

All of the preparations were made according to the process described; the initial activity of each was indicative of an active preparation. There is no uniformity of results; O_1 and S_2 are active even though they are over a year old, and have had no special treatment to prevent destruction.

On the other hand A_2 , M , L_1 , have lost their activity no matter whether kept in a sealed tube or under toluol in the ice box.

Instability in acid solution. In all the preparations 0.4 per cent HCl was used, and in each case the secretin was made neutral to litmus before it was injected, to test its activity.

The stability of secretin in acid solutions. Secretin C had such an activity that when 4 cc. were injected there was a flow of 22 drops of pancreatic juice in 10 minutes.

TABLE 4
Instability of secretin on standing

PREPARATION	DATE PREPARED	DATE TESTED	ACTIVITY (DROPS PAN- CREATIC JUICE IN 10 MIN- UTES)	REMARKS
A_2	November 17, 1921	{ November 23, 1921 November 27	15 1	Kept in sealed tube
O_1	February 11, 1922	{ February 13, 1922 August 15, 1923 July 2, 1923 July 2, 1923	17 11 7 9	Kept in ice box Kept in ice box Kept in sealed tube
M_1	March 3, 1922	{ March 4, 1922 March 17, 1922	22 2	Kept in ice box Kept in ice box
S_2	March 8, 1922	{ March 10, 1922 August 15, 1922 July 2, 1923	18 15 10	Kept in ice box Kept in ice box Kept in ice box
L_4	April 24, 1922	{ April 24, 1922 August 15, 1922	23 2	Kept in sealed tube
M_3	August 8, 1922	{ August 15, 1922 July 2, 1923	30 18	Kept in sealed tube

The same secretin evaporated to dryness on the steam bath, made up to volume again with water, when injected causes only a small flow of 7 drops of juice in a 10-minute interval.

Four cubic centimeters of secretin D when injected stimulated a flow of 40 drops of pancreatic juice in 10 minutes.

After concentrating in vacuo, then making up to the original volume, the injection of 4 cc. instigated a flow of 44 drops of pancreatic juice in 10 minutes.

When the secretin was heated on the steam bath with a reflux (condenser) for 6 hours, there was a loss of activity. The injection of 4 cc. caused a flow of 7 drops in 20 minutes.

Similar results were obtained with four other preparations of secretin. Evaporation of a secretin solution in acid, out in the air, causes it to lose two-thirds of its activity. Boiling with a reflux condenser causes it to lose about two-thirds of its activity also. Concentration in vacuo at 50 to 60°C. does not destroy the activity of secretin, whether it is concentrated with 0.4 HCl or whether it is neutralized before concentration.

Instability in alkaline solutions. The most active secretin preparations are those in which the acidity is kept about 0.4 per cent HCl, and in which the material at no time becomes alkaline, or if so, for only a very short time. Boiling with alkali even with dilute alkali, completely destroys the activity. A secretin solution which stands in a strong NaOH solution over night loses its activity.

A secretin preparation which is made only slightly alkaline to litmus, does not lose its activity entirely but to some extent. The alkalinity

TABLE 5

The instability of secretin in alkaline solutions

PREPARATION	ACTIVITY (DROPS PANCREATIC JUICE)	TREATMENT
Secretin Y	30 (in 15 minutes)	4 cc. of the unconcentrated secretin was injected intravenously
	1 (in 30 minutes)	After boiling with 1 per cent NaOH 6 hours. Neutralized before injection
	0 (in 30 minutes)	After standing in 29 per cent NaOH over night
	12 (in 15 minutes)	After being N/10 alkaline for 6 hours
	15 (in 15 minutes)	After treatment with lime in 50 per cent alcohol, concentrated in vacuo

present when lime in excess is added to an acid solution in 50 per cent alcohol lowers the activity but does not destroy it. The concentration of alcohol (2 grams NaOH to 10 cc.) in the solution from which histamine is extracted with amyl alcohol, is sufficient to destroy the secretagogue action of a secretin solution.

Typical results are tabulated in table 5.

In each case 50 cc. of the secretin was treated as described, and the amount injected was concentrated so that the original volume remained. The neutralized solutions were injected intravenously.

Instability of the phosphotungstic acid precipitate of secretin. The loss of the secretagogue action of secretin preparations has been brought out in the analysis of the preparation for histamine. The physiological assay of the fractions showed that this loss occurred after the solution was treated with phosphotungstic acid, and that the action could not be recovered from the precipitate, nor from the filtrate.

A secretin preparation which was neutral after having been concentrated in vacuo was made slightly acid and precipitated with phosphotungstic acid. The precipitate was decomposed by HCl and the phosphotungstic acid removed by an immiscible solvent of equal parts of amyl alcohol and ether.

Similar results were obtained from two other equally active preparations of secretin.

Instability in amyl alcohol: Secretin cannot be extracted from an alkaline solution by amyl alcohol. When an extract of duodenal mucosa is treated with amyl alcohol, the secretagogue action is neither extractable from the alcohol by acid treatment, nor does it remain in the alkaline water phase.

Extractions with amyl alcohol were made of secretin preparations, the alkalinity of which was varied, and the destructive effect of the alkali alone was noted.

TABLE 6

The instability of secretin precipitated by phosphotungstic acid

PREPARATION	ACTIVITY (DROPS PANCREATIC JUICE)	TREATMENT
Secretin J	20 (in 10 minutes)	1 cc. of concentrated secretin
	1 (in 10 minutes)	P. T. A. precipitate decomposed by acid, concentrated, neutralized
	0 (in 30 minutes)	Filtrate from the precipitate concentrated, neutralized

Typical results on a number of different preparations of secretin, having been variously treated, are as follows:

When 2 cc. of concentrated secretin were injected intravenously there was a fall of blood pressure from 110 to 40 mm. of mercury and a flow of 24 drops of pancreatic juice in ten minutes.

A portion of the same secretin which had been made alkaline with 0.4 per cent NaOH, kept alkaline for 4 hours, then neutralized and concentrated to the original volume, when injected showed a small decrease in activity. There was a fall in blood pressure from 100 to 48 mm. of mercury, and a flow of 15 drops of pancreatic juice in 15 minutes.

Some of the alkalized secretin preparation was extracted with amyl alcohol, and recovered from the amyl alcohol with 0.4 per cent HCl, then concentrated to the original volume. When injected it caused a fall in blood pressure from 100 to 60 mm. Hg, but there was no flow of pancreatic juice.

The solution remaining after the amyl alcohol extraction, caused a fall in blood pressure from 100 to 68 mm. Hg but no stimulation of pancreatic juice.

Secretin which was kept more strongly alkaline, with 1 per cent NaOH for 4 hours, then neutralized and concentrated to volume, caused a fall of blood pressure from 104 to 50 mm. Hg and a secretion of 10 drops of pancreatic juice in 10 minutes.

Amyl alcohol extraction from the 1 per cent NaOH solution of secretin, and recovery with 0.4 per cent HCl, left a solution which caused a fall in blood pressure from 98 to 52 mm. Hg, but no stimulation to pancreatic flow.

The solution remaining after the extraction caused a very slight fall in blood pressure from 98 to 80 mm. Hg and no flow of pancreatic juice.

Secretin which was made very strongly alkaline with 20 per cent NaOH for 4 hours, then neutralized and concentrated to volume, caused a decrease in blood pressure from 106 to 52 mm. Hg, but there was no stimulation to pancreatic flow.

This 20 per cent alkaline solution extracted with amyl alcohol, recovered with 0.4 per cent HCl neutralized, concentrated and injected produced a fall in blood pressure from 102 to 50 mm. Hg, but there was no flow of pancreatic juice following.

The solution remaining after the amyl alcohol extraction, contained neither depressor substance nor secretagogue action.

The results show that alkalinity is an important factor in destroying secretagogue action. The alkalinity of 0.4 per cent NaOH, and 1 per cent NaOH did not destroy the activity but reduced it considerably, the 1 per cent NaOH more than the 0.4 per cent. The 20 per cent NaOH completely destroyed the activity.

In all instances the secretagogue activity was destroyed by amyl alcohol extraction. Amyl alcohol extracts the depressor substance from a strongly alkaline solution also.

Secretin is extracted from a solution by Fuller's earth. Secretin is taken out of a solution by treatment with Fuller's earth, and can be recovered by extraction of the earth by treatment with acid. Fuller's earth which has been saturated with NaCl, does not remove the secretin.

This indicates that secretin is a basic substance, which is taken up by the Fuller's earth. Fuller's earth here acts as does permutit in absorbing the ammonia from solutions.

Stability of secretin after freezing. Ground duodenal mucosa was frozen for 24 hours at -18°C . It was then chopped in the Kossel machine and the juice pressed out by a hydraulic press. This was made acid, the proteins which precipitated out were filtered off, the solution neutralized and made up to volume, so that 1 cc. represented 4 grams of the mucosa. A control secretin was made with a portion of the ground duodenum in the usual way, by heat extraction with HCl.

The action of freezing on secretin. The control secretin made in the

usual manner when injected, produced a fall in blood pressure from 120 to 54 mm. Hg, and stimulated a flow of 32 drops of pancreatic juice in 20 minutes.

Secretin prepared from ground-up frozen duodenum showed similar results, a fall of pressure from 134 to 64 mm. Hg, and 29 drops of pancreatic juice in 20 minutes.

Secretin prepared from the pressed juice exhibited about the same activity, a fall in pressure from 136 to 54 mm. Hg, and 30 drops of pancreatic juice in 20 minutes.

The secretagogue action of the secretin from the frozen mucosa and from the pressed juice is as great as the original secretin, prepared in the ordinary way. If the activity were due to intracellular substances, it should have increased when the cells were disrupted in the process of freezing.

TABLE 7
Secretin treated with Fuller's earth

INJECTION AND TREATMENT	BLOOD PRESSURE			PANCREATIC SECRETION	
	Normal	Low	Fall	Drops	Time
1 cc. concentrated secretin.....	96	40	56	27	minutes 10
100 cc. concentrated secretin treated with Fuller's earth. Recovered by pressing out the juice.....	94	58	36	27	10
Fuller's earth remaining treated with 0.4 per cent HCl.....	98	50	48	20	10
Concentrated secretin treated with earth saturated with NaCl previously.....	100	50	50	18	10

Quantitative comparison of the physiological activity of secretin and histamine. Histamine and secretin, as ordinarily prepared, both possess depressor action and secretagogue action. Popielski attributes the two actions to "vasodilatin" which he considers a constituent of most tissue extracts and suggests the similarity to histamine.

The separation of the depressor action and the secretagogue action is a very difficult matter. Bayliss and Starling tried to prepare a secretin free from vasodilatin by extracting the depressor substance with absolute alcohol, but their protocols show that with a decrease in depressor effect, there is also a decrease in the secretory action. Matsuo (19) found that the acid which had remained in the duodenum for a short time, 5 to 10 minutes, contained a substance which when introduced intravenously caused a secretion from the pancreas without a fall in blood pressure. However, if the acid remained longer in the duodenum, 30 to 45 minutes, it contained a substance which not only caused a secretion, but also a

depression of the blood pressure. This work has been confirmed recently by Luckhardt and Grogan (16).

Depressor substances do not cause a secretion of pancreatic juice necessarily. Arthus (17) tried a variety of substances which produced a fall in blood pressure and anaphylactic shock, but found that they did not cause a secretion of the pancreas, nor augment a secretion which was normal. Preparations of duodenal mucosa retain their depressor effect after their secretagogue action has disappeared.

The experiments of previous workers have been carried out on animals under ether anesthesia, so that the results may be due to variations in the anesthesia and the asphyxia of the cells due to the anesthesia. Under the barbital anesthesia which has been used in these experiments, the blood pressure varies within such narrow limits that the depressor effects can be quantitatively measured. The secretagogue action of a histamine solution, which produces the same fall in blood pressure as a secretin preparation, can be compared with the secretagogue action of the same secretin solution.

Physiological action of histamine and secretin. The injection of 2 cc. secretin caused a fall in blood pressure of 66 mm. Hg, a drop from 96 to 30 mm. The return to normal occurred after 12 minutes. After a latent period of 75 seconds, there were 28 drops of pancreatic juice within the next 12 minutes after which the flow was normal.

The injection of 0.0001 gram of pure histamine produces a fall of 62 mm. Hg, from 90 to 28, with a return to the normal height in 6.5 minutes. Following a latent period of 100 seconds there is a flow of 4 drops of pancreatic juice in 12 minutes.

The injection of a large dose of histamine, namely, 0.00015 gram, caused a drop of 64 mm. in the blood pressure, from 100 to 36, which returned to the normal height in 6 minutes. After a period of 130 seconds, there was a flow of 6 drops of pancreatic juice during the next 12 minutes.

To show that the secretory mechanism was intact, 2 cc. of secretin used above were injected which caused a fall of 66 mm. Hg, from 98 to 32, with a return to normal in 12 minutes 60 seconds. After the injection there were 23 drops of pancreatic juice in 12 minutes.

Increasing doses of pure histamine (HCl)₂ were injected: 0.0002 gram caused a fall of 62 mm. Hg and a flow of 4 drops of pancreatic juice; 0.0003 gram produced a drop of 66 mm. Hg, and a secretion of 6 drops of pancreatic juice; 0.0006 gram resulted in a fall of 72 mm. in the blood pressure and a flow of 11 drops of pancreatic juice; 0.0012 gram injected produced a fall from 112 to 30 mm. Hg, a fall of 82 mm. There were only 8 drops of pancreatic juice after a latent period of 140 seconds.

Deep intramuscular injections of histamine failed to cause any fall in the blood pressure. Deep intramuscular injections of secretin likewise

produce no effect on blood pressure. Neither causes a flow of pancreatic juice. (In other experiments it has been shown that while there is no effect on the pancreatic secretion, the intramuscular injection does cause a flow of gastric juice.)

From the blood pressure tracing and the tables one can draw the following conclusions:

1. The blood pressure tracing obtained after the injection of secretin is very similar to that obtained after the injection of histamine. In each case there is a quick initial fall followed by a slight rise after which there is a secondary fall followed by a steady rise until there is a return to normal. The return to normal is more prompt after the injection of histamine than it is after secretin. Even large doses of histamine which cause very great depression return to the normal level very soon, in most cases after about 5 minutes. Secretin, which depresses to the same amount requires a longer time to return to normal.

2. The number of drops of pancreatic juice is increased with secretin much more than after an injection of histamine, 28 drops after secretin as compared with 4 to 6 drops after histamine, in the same length of time, and with the same fall in blood pressure. That is, histamine is the less efficient secretagogue, but the more powerful depressor.

3. The time from the injection of the substance to the initial drop of juice is longer with injections of histamine (the latent period in the table).

4. Very large amounts of histamine did not augment the secretion from the pancreas much more than a dose one-tenth the amount. In no case was the flow comparable to that caused by secretin.

5. Finally, histamine itself is not responsible for the "secretin" action which causes the flow of pancreatic juice, although there is a histamine-like action in the effect on the blood pressure.

Effect of introducing secretin and histamine into the duodenum. Secretin solutions were prepared as for the previous experiments, and histamine solutions were made in NaCl of the same strength as the salt content of the neutralized secretin preparations. In addition preparations of gastrin, prepared from the gastric mucosa in exactly the same way as the secretin, were used.

The dogs were under barbital anesthesia. The pancreatic duct was cannulated, the pylorus tied off, the bile duct ligated, as well as the accessory pancreatic ducts. A loop of the intestine including the duodenum and a small part of the jejunum was isolated and a cannula placed in each end.

Blood pressure tracings were made from the carotid artery. The intravenous injections were made into the saphenous vein.

In order to control all factors and to be sure that the secretory mechanism was intact, the following order of experimentation was carried out:

Outline of the experiment. 1. Control on the activity of the pancreatic mechanism by the intravenous injection of secretin of known value.

2. Determine the minimum dose of the secretin to be tested which gives similar results.

3. Determine the minimum dose of histamine which reduces the blood pressure to the same amount as the secretin.

4. Test the activity of the gastrin as to its effect on blood pressure and pancreatic secretion.

5. Wash out the intestinal loop with 50 cc. of normal salt solution.

6. Introduce 50 cc. neutral secretin into the loop and allow to remain 30 minutes, while observing the blood pressure and the rate of pancreatic secretion. Test the recovered material for its action by intravenous injection.

7. Introduce 50 cc. histamine solution into the loop, watch the effects for 30 minutes, remove and inject some of the recovered material intravenously.

8. Repeat with neutral gastrin.

9. Test the absorbent ability of the intestinal loop by the introduction of 50 cc. 0.4 per cent HCl.

10. Retest the secretory mechanism by the intravenous injection of the same secretin of known value.

RESULTS. Three different dogs were used with three different preparations of secretin and gastrin. The results in general are very similar.

When the material was removed from the loop, more than the original 50 cc. introduced were recovered in spite of the fact that the salt content was that of a physiological solution. The loop was washed with 0.9 per cent NaCl and the washings added to the filtrate, the total volume being made up to 100 cc. Since this was twice the amount introduced, the intravenous injection made was with 2 cc., twice the volume of the original secretin solution injected. In this way all results were made comparable.

The results on one of the animals are sufficient to show the typical findings.

When 50 cc. secretin were introduced into the loop, 65 cc. were recovered at the end of 30 minutes.

When 50 cc. histamine solution were injected, 70 cc. were recovered.

After the introduction of 50 cc. gastrin for 30 minutes, 75 cc. were recovered.

Absorption of secretin from the duodenum. One cubic centimeter of secretin injected intravenously produced a fall from the normal blood pressure of 110 to 50 mm. Hg, a fall of 60 mm. The blood pressure returned to normal in 7 minutes 7 seconds. This injection stimulated a flow of pancreatic juice of 15 drops in 7½ minutes. The secretin injected above was a stable solution, which had been used in a number of experiments as a control on the animal preparation.

This was compared with the injection of 1 cc. of the secretin used in this experiment in which there resulted a fall of blood pressure from 112 to 50 mm. Hg with a return to the normal height in 6 minutes 20 seconds. This stimulated a flow of 13 drops of pancreatic juice in $7\frac{1}{2}$ minutes.

The injection of 0.0001 gram of histamine intravenously caused a reduction in the blood pressure from 114 to 48 mm. Hg, with a return to normal in 2 minutes 38 seconds. From this injection, 5 drops of pancreatic juice resulted in 7-minutes.

One cubic centimeter of gastrin injected intravenously, caused a fall of blood pressure from 110 to 58 mm. Hg, with a return to normal after 5 minutes. There was no stimulation of pancreatic activity, three drops being formed in 10 minutes, which is but slightly more than normally noted.

When 50 cc. of 0.9 per cent NaCl were introduced into the duodenal loop, there was no fall from normal blood pressure of 110 mm. of mercury, and during the 30-minute period it remained in the loop there was no change in the rate of secretion of pancreatic juice.

The introduction of 50 cc. of secretin into the loop caused no change in the normal blood pressure of 105 mm. Hg. It caused no change in the rate of secretion of the pancreas. The secretin was removed from the loop, the loop washed out with 0.9 per cent NaCl, and the recovered secretin and washings made up to 100 cc.

The injection of 2 cc. of this material intravenously produced a fall in blood pressure from 112 to 48 mm. Hg, with a return to the normal height in 2 minutes 37 seconds. There was only a slight stimulation of pancreatic secretion, four drops being recorded in 6 minutes.

When 50 cc. containing 0.0001 gram histamine were introduced into the intestinal loop, there was a change in the blood pressure from 104 to 95 mm. Hg, but no typical fall. There was no stimulation to pancreatic activity.

The loop containing the histamine was emptied and rinsed out, the rinsings being added to the recovered histamine. Two cubic centimeters were injected intravenously producing a fall in blood pressure from 95 to 38 mm. Hg, with a return to normal in 5 minutes 10 seconds. There was no noticeable effect on the rate of pancreatic secretion following the injection.

Fifty cubic centimeters of gastrin in the duodenal loop caused no change in the blood pressure. There was no secretion of pancreatic juice during the 30 minutes it remained in the duodenal loop.

The gastrin was recovered from the loop and 2cc. injected intravenously. The blood pressure fell from 108 to 60 mm. Hg with a return to normal in 3 minutes 20 seconds. There was no stimulation to pancreatic secretion.

To show that the loop was capable of absorption and that the animal

was in good condition, 50 cc. of 0.4 per cent HCl were placed into the duodenal loop. There was a very small decrease in blood pressure from 95 to 90 mm. Hg, with a return to normal in 5 minutes, after the mechanical effects had worn off. On the other hand, this 0.4 per cent HCl caused a flow of pancreatic juice of 16 drops in 10 minutes. The stimulation began after the 0.4 per cent HCl had been in the loop 20 minutes.

Following all these experiments, the injection of 1 cc. of the standard secretin previously used caused a fall of 55 mm. Hg, and after a 45 second latent period, there was a flow of 10 drops of pancreatic juice in 7 minutes.

The whole series of experiments carried out as described above was repeated on two other dogs with two different samples of secretin. The results were in every way comparable to the ones just described, and add nothing new to the evidence.

SUMMARY. From the results it is seen that:

1. There is no secretagogue action on the pancreas when secretin, histamine, gastrin or normal salt is in the duodenum. Hydrochloric acid (0.4 per cent) in the duodenum, however, causes the usual marked secretion of the pancreas.

2. There is no change in the blood pressure when secretin, histamine, gastrin, normal salt solution or 0.4 per cent HCl is in the duodenum. Hydrochloric acid acts as a secretagogue without lowering the blood pressure.

3. Both secretagogue and depressor actions are present in the histamine, secretin and gastrin solutions when removed from the duodenum, after having been in the lumen for 30 minutes. The depressor action is possibly very slightly reduced, but the secretagogue action is reduced very decidedly.

DISCUSSION. The fact that the neutral secretin is not absorbed from the intestine confirms the findings of Bayliss and Starling (1), Wertheimer (18) and Matsuo (19) as well as others. That the negative results are not due to the destruction in the intestinal loop is demonstrated by the physiological activity of the recovered material. It was expected that the recovered material would be more active than the original, in view of the fact that aqueous extracts of the duodenum may cause a slight secretion when injected intravenously. The opposite was actually found; there was neither absorption of the neutral material nor extraction of a more active substance from the duodenum. The most obvious conclusion, then, is that there is a physiologically active substance present in the neutral preparation, which if absorbed becomes inactive. Neither is there a substance formed by the neutral extraction of the living duodenum, as shown by the diminished activity of the recovered material, nor which is carried away unaltered by the blood stream, as shown by the negative response of the blood pressure and the secretin while the material was in the loop.

That histamine is not absorbed unaltered is contrary to what was expected in view of the work of Gerard (20) in which he finds the presence of histamine in the loop fluid formed in washed segments of the upper jejunum, the toxic effect of which caused the death of the animal. Symptoms of toxemia developed after 24 hours; on autopsy the intestinal mucosa was found to be necrotic. However, the same worker shows that histamine may be in the closed loop of the colon and cause no toxic symptoms. It appears that factors other than histamine content are involved in the toxemia from closed intestinal loops.

That neither secretin nor histamine is absorbed unaltered from the small intestine indicates that they have some properties in common, but it does not necessarily prove that they are identical. It is probable that neither secretin, as it is usually prepared, nor histamine alone represents the substance (if any such exists) which is responsible for the physiological process of the stimulation of the pancreatic glands during digestion.

GENERAL CONCLUSIONS

1. Secretin solutions prepared from dog duodenal mucosa do not contain histamine in amounts which are of physiological significance.
2. Secretin preparations do not possess their depressor and secretagogue actions as a result of their histamine content.
3. Secretin and histamine are similar, but by no means identical chemically. The latter is much more stable; the former is not precipitable by phosphotungstic acid in active form; also, it is insoluble in amyl alcohol and disappears from the alkaline aqueous solution which was extracted with the amyl alcohol.
4. Secretin and histamine are similar, but by no means identical physiologically. Histamine is the less efficient secretagogue, but the more powerful depressant.
5. Neither secretin nor histamine appear to be absorbed unaltered from the duodenal loop and neither appears to be destroyed therein. This has been shown by physiological assays. Therefore, even if secretin were formed in the natural digestion in the manner claimed by Bayliss and Starling, it would not be absorbed unaltered from a neutral solution.
6. The secretin preparations usually injected do not appear to represent a true physiological process.
7. The losses in secretagogue action as compared with no loss in depressor action observed in the various destructive treatments applied to secretin preparations suggest that the two activities are due to two different substances or different states of the same substance.

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